CHAPTER 1

INTRODUCTION

This chapter introduces the experimental work described in the following chapters. This work includes the study of the influence of four classes of additives on a number of properties of cationic vesicles. Particular attention is placed on the *catalytic* properties of these vesicles on a model deprotonation reaction. The results possess relevance for understanding non-enzymatic reactions proceeding at the polar-apolar interface of biological membranes, since biological membranes are complex mixtures of a wide variety of compounds.

In this chapter driving forces for surfactant aggregate formation in water will be discussed first with a particular focus on vesicles, since they can serve as mimics for biological membranes. Then the composition and some properties of biological membranes will be briefly discussed. A comparison between the properties of biological membranes and vesicles formed from synthetic amphiphiles will be made. Then an introduction into vesicular catalysis will be presented including important differences between catalysis in micellar and vesicular solutions. Finally, the aims of this thesis are outlined.

1.1 Water and Aggregation Processes in Water

1.1.1 Hydrophobic Hydration

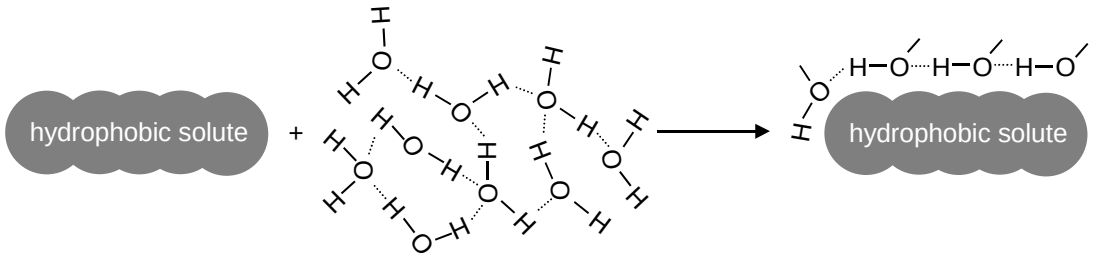
Water is a special liquid due to its low molecular weight (and hence small molar volume) it has a high melting temperature and a high boiling temperature.[1,2] These properties are a result of a, for such a small molecule unique, three-dimensional hydrogen bond network, based on two hydrogen-bond donating and two hydrogen-bond accepting sites. The high heat capacity of water is related to this hydrogen-bonding ability. Another feature of the 3-D hydrogen bond network is that apolar molecules, such as hydrocarbons, have a limited solubility in water. Unlike, for example, glucose, hydrocarbons fit only poorly into the "structure" of water ("hydrophobic hydration"). As a result, the Gibbs energy of transfer of hydrocarbons from the gas phase to water is large and positive. For example, at 25°C the Gibbs energy of solvation ($\Delta_{solv}G$) of gaseous methane into a series of organic solvents (*n*-hexadecane to methanol) varies between 12.7 and 17.5 kJ mol^{-1}, whereas $\Delta_{solv}G$ in water is 25.5 kJ mol^{-1}.[3] Contrary to what might be anticipated on the basis of the large Gibbs energy, interactions between water and organic substrates are not unfavourable. In fact, London dispersion interactions between water and apolar solutes are quite favourable.[4]

At room temperature $\Delta_{solv}H$ of apolar gasses in water is favourable. However, the entropic contribution is much more unfavourable than that the enthalpy is favourable, and hence, the Gibbs energy is positive. Interestingly, as the surface area of the apolar molecule increases, the enthalpy decreases, but at the same time the entropy ($T\Delta_{solv}S$) decreases to almost the same extent, leading to $\Delta_{solv}G$ that is only weakly dependent on the size of the

apolar solute. In contrast, $\Delta_{solv}G$ of the same molecules in *n*-hexane decreases upon an increase in surface area, due to a decrease in enthalpy that is larger than the loss in entropy.

In addition, the heat capacity of transfer of apolar molecules to water is large and positive, leading to unfavourable enthalpic contributions at higher temperatures. However, since hydrogen bonds are progressively broken upon increasing the temperature, the entropic contribution decreases, and hence the Gibbs energy is almost constant with temperature.[5] As a consequence, at room temperature the large positive Gibbs energy originates from a large unfavourable entropic contribution, whereas at higher temperatures it results from a large unfavourable enthalpic contribution. Polar groups generally have a heat capacity that is slightly negative. Interestingly, the ability of arenes to form "weak" hydrogen bonds leads to a negative Gibbs energy.[6,7]

In conclusion, the large and positive heat capacity, the large entropic contribution at room temperature, the large enthalpic contribution at higher temperatures, and the poor solubility of organic substrates in water is what distinguishes hydrophobic hydration from other solvation effects.[8-12]



Scheme 1.1. *Schematic representation of the change in the three-dimensional hydrogen-bond network as a result of the hydration of an apolar solute.*

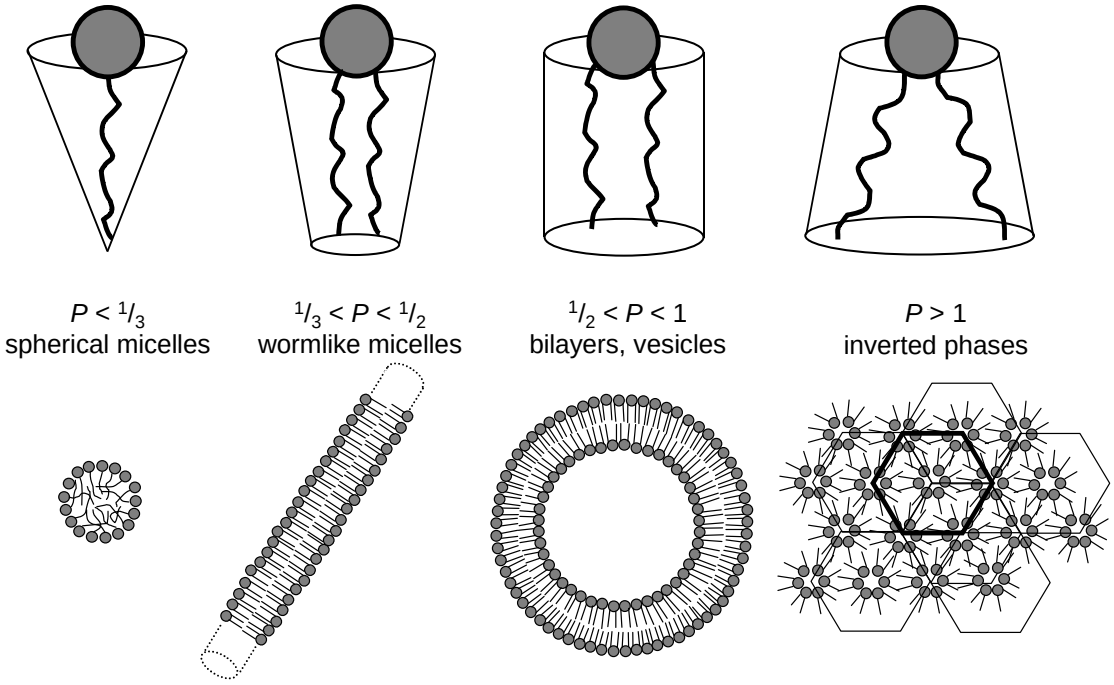
In 1945 Frank and Evans proposed the existence of iceberg-like water structures around apolar solutes.[13] In this model, water molecules are highly ordered in an ice-like structure. The unfavourable entropy term was connected to the formation of highly structured water, involving stronger or more hydrogen bonds in the hydrophobic hydration shell as compared to bulk water. This idea was widely accepted for some time until experiments started to cast doubt on this theory. Since then there have been many debates in the literature about the physical origin of hydrophobic hydration.[14-21] In the past few years, it has been accepted that in the hydrophobic hydration shell water molecules are oriented in such a way that a maximum number of hydrogen bonds is preserved.[17-21] This is the case when the water molecules have preferentially one of their hydrogen-oxygen bonds oriented tangential to the hydrophobic surface (Scheme 1.1).

1.1.2 Hydrophobic Interactions and Colloidal Aggregates

1.1.2.1 Important Parameters

When the hydrophobic hydration shells of two apolar solutes in water overlap, water molecules are released into the bulk solution. As a result, at room temperature this process is driven by a favourable change in entropy. Since this process is essentially a reversal of

hydrophobic hydration, similar thermodynamic trends are observed. Above a certain concentration of solute, the apolar solutes start to interact extensively, accompanied by a release of water molecules. Depending on the nature of the solute(s) this can lead to several type of processes. These can be divided into pairwise (1:1) interactions, interactions involved in small aggregates ("moving units"; aggregation number between 3 and 10) and bulk interactions. Examples of pairwise interactions are enzyme-inhibitor interactions[22] and hydrotrope complexes.[23] Examples of interactions playing a role in the formation of "moving units" occur when short (C_2-C_7) alcohols are dissolved into water.[24] Bulk interactions are involved in phase separation and colloidal aggregation.[25] Examples of the former are observed in mixtures of water and hydrocarbons. Above a threshold concentration the mixture becomes oversaturated in hydrocarbons and as a result forms a two-phase system. Molecules bearing both a polar and an apolar group may separate on a microscopic scale when dissolved in water. The mechanism and driving forces are similar as for hydrocarbons. However, transfer of a polar group from water to an organic solvent(-like) phase is highly unfavourable.[26,27] Therefore, macroscopic phase separation is unfavourable and hence the molecules reorganise in such a way that the favourable interactions of the polar groups with water are largely retained. As a result different types of colloidal aggregates are formed (Scheme 1.2).



Scheme 1.2. *Schematic representation of structures that can be formed as a function of the packing parameter.*

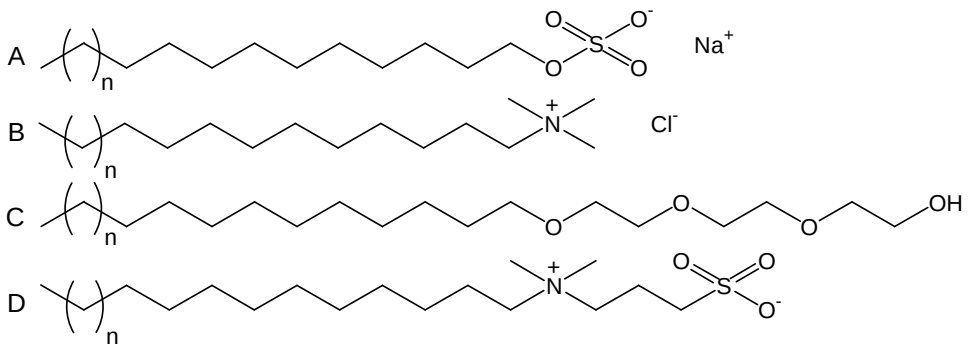
Molecules that form these aggregates are known as surfactants (surface active agents) or amphiphiles ("αμφι φιλος" means "loved on both sides" in ancient Greek). The exact morphology of the aggregate is determined by a subtle interplay between the nature of the polar group and the size of the apolar group.[28,29] The packing parameter *P*, developed by Israelachvili and Ninham, gives a prediction of the structure of the aggregate that is formed:[30]

$$P = \frac{V}{a_0 l_c} \tag{1.1}$$

In this equation V is the volume of the hydrophobic part of the molecule, a_0 the mean cross-sectional head group surface area and l_c the length of the extended all-trans alkyl tail. As can be seen in Scheme 1.2, when the packing parameter is small (< $^1/_3$) the shape of the surfactant favours a large positive curvature, leading to small aggregates (micelles), whereas when the packing parameter is large (>1) there is a negative curvature leading to inverted structures (*e.g.* hexagonal, cubic, etc.). In between these extremes various structures may form, such as worm-like micelles, (flat) bilayer fragments and vesicles. Under certain circumstances also two different types of aggregates can coexist.
In the following two subparagraphs micelles and vesicles will be discussed in more detail, since they are relevant for understanding the observations described in the other chapters of this thesis. Description of other structures and their properties can be found in an excellent book by Evans and Wennerström.[25]

1.1.2.2 Micelles

Spherical micelles are usually formed from surfactants with a single alkyl tail that contains between 8 and 18 carbon atoms. The head group may be cationic, anionic, zwitterionic, or nonionic. Molecules with shorter alkyl tails, such as hydrotropes, form short-lived non-micellar aggregates. Longer alkyl tails cause the crystal packing to be more favourable than the solubilisation process into water. The concentration, above which surfactants aggregate into micelles, is called critical micelle concentration (cmc). The cmc is typically in the order of 10^{-6} to 10^{-2} M. Above the cmc the solution consists of micelles and monomers. The monomer concentration roughly equals the cmc. Due to the lack of ionic repulsion, the cmc of nonionic surfactants is typically 1-2 orders of magnitude lower than that for typical ionic surfactants. Increasing the size of the tail decreases the cmc. Due to the binding of counterions to the surface, the head group repulsion will be reduced and the high charge density in the polar-apolar interface of micelles (the Stern region) will be lowered. The counterion binding is typically in the order of 70-90 %.



Scheme 1.3. *Examples of an anionic, a cationic, a nonionic and a zwitterionic micelle-forming surfactant (top to bottom). Sodium n-alkylsulfate (A); n-alkyltrimethyl-ammonium chloride (B); tri-ethylene glycol mono-n-alkyl ether (C); n-alkyldimethyl-propanesultaine (D). Typically n ranges from 1 (n-dodecyl) to 7 (n-octadecyl).*

Micelles are dynamic and relatively small aggregates (diameters around 5-6 nm).[31] Their average lifetime is in the order of milliseconds, but monomers enter and leave the aggregate on the microsecond timescale.[32] The polar head groups are on the outside, and the inside of the micelle consists of hydrocarbon chains that are largely in the liquid phase.[28] Typically, the number of molecules in a micelle is about 50-100, although there are many exceptions to this rough estimate. Some examples of micelle-forming surfactants are shown in Scheme 1.3.

At surfactant concentrations above 50-100 mM, interactions between individual spherical micelles start to become important. As a result, long worm-like micelles are formed. The packing parameter no longer predicts the correct aggregate morphology. Due to these elongated structures the viscosity of the solution increases.

1.1.2.3 Vesicles

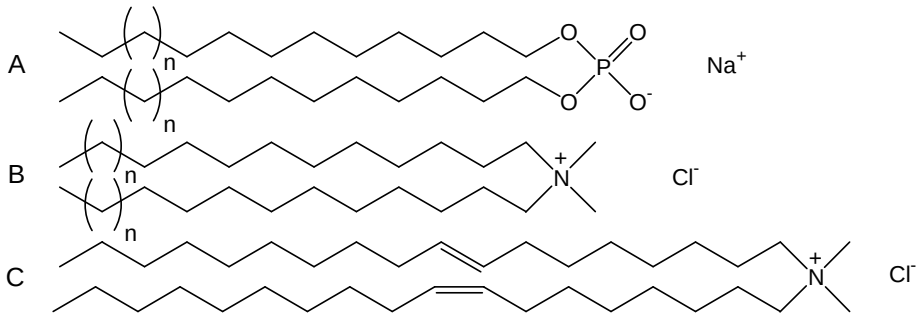
Vesicles are usually formed from amphiphiles containing two alkyl tails (Scheme 1.4). Similar to micelles, vesicles are also formed above a critical concentration (critical vesicular concentration; cvc). Due to the doubling of the number of carbon atoms, the cvc is generally lower than the cmc of corresponding single-tailed micelle-forming surfactants (*e.g. n*-hexadecyltrimethylammonium bromide versus di-*n*-hexadecyldimethylammonium bromide). The morphology of vesicles is significantly different from the micellar structure. Vesicles consist of a double layer of surfactants that entraps an aqueous compartment (Scheme 1.2). This means that the outer leaflet has a positive curvature, and the inner leaflet a negative curvature.

Due to a decrease in curvature, vesicles have a diameter that that can range from 30 nm up to 10 µm. The average size and size distribution will depend on the method of vesicle preparation.[33] In the past a popular procedure was the so-called ethanol or chloroform injection method.[34-37] In this procedure the amphiphile is dissolved in a small amount of ethanol or chloroform in order to obtain a homogeneous solution. Then, a small volume is injected into water, usually followed by heating of the aqueous solution in order to evaporate the organic solvent. However, this latter process does not necessarily remove all the organic solvent from the mixture. Although the volume percentage of the organic solvent is usually small,[38] the effect on the resulting vesicles can be large.[39,40] Particularly the addition of ethanol can lead to undesired behaviour or properties.[40-44] These procedures are often used in order to obtain vesicles with a well-defined size (distribution). Nowadays other procedures are known to make vesicles with a well-defined size without the disadvantage of potentially having residual organic solvent in the vesicular solution. Small vesicles (*ca.* 30 nm in diameter) can be obtained by dissolving the amphiphiles in water, followed by extensive tip-sonication above the main phase transition temperature. Larger vesicles, up to several micrometers, can be obtained by dissolving the amphiphile in a small amount of an organic solvent, and subsequently evaporating the solvent by slowly rotating the tube containing the solution under a stream of nitrogen. In this way a slowly formed thin film of amphiphile is formed. After leaving this film under reduced pressure for several hours, all the organic solvent is evaporated. Then the film can be hydrated by addition of the appropriate amount of water. Repeatedly freezing the solution in liquid nitrogen and thawing it in warm water yields large multilamellar vesicles. If these vesicles are then

pushed several times through a membrane with a well-defined pore size, vesicles with that particular size are obtained.[31,45]

Due to the morphological differences compared with micelles, vesicles have different dynamics and stability. In addition, vesicles have a number of properties that are unknown for micelles, such as the phase of the tails, the permeability of the membrane towards ionic and nonionic molecules and the vesicle size and shape. In the following section these properties will be addressed in more detail.

Vesicles formed from a non-equimolar mixture of cationic and anionic (single-tailed) surfactants are thermodynamically stable, but vesicles formed from other surfactants or surfactant mixtures are metastable.[46,47] Hence, these latter vesicles precipitate with time, although the rate of this process varies between seconds and months. This approximately 10^7-fold time difference is the result of a very complex interplay of several processes that are involved in the precipitation process. Usually, with time vesicles aggregate, fuse and precipitate, or the tails crystallise, after which the bilayer fragments precipitate. Both processes can occur simultaneously as well. Fusion can be minimised using charged vesicles, or vesicles that are sterically stabilised. However, these processes depend on many more parameters, such as temperature, vesicle size and vesicle preparation method.[32,48,49]

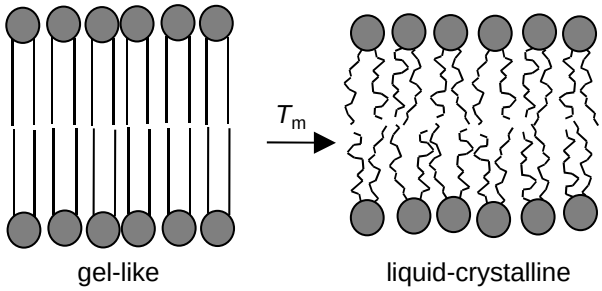


Scheme 1.4. *Examples of an anionic and two cationic vesicle -forming surfactant (top to bottom). Sodium di-n-alkylphosphate (A); di-n-alkyldimethylammonium chloride (B); dimethyldioleylammonium chloride (C). Typically n ranges from 1 (n-dodecyl) to 7 (n-octadecyl).*

Within the bilayer the alkyl tails can exist in phases that differ in their fluidity. At low temperatures the tails are in a highly ordered, rigid (gel-like) state (Scheme 1.5). Above a critical temperature (T_m) the tails are transformed into a more fluid (liquid-crystalline) state. In this state the tails have the freedom to adopt a large variety of conformations. Many of the vesicular properties depend on the phase of the tails, such as permeability, microviscosity, the ability to bind small molecules, susceptibility to pore formation, the extent of water penetration, amphiphile diffusion and vesicle fusion.[50] For example, below the T_m the microviscosity decreases with temperature. At the T_m the microviscosity makes a jump downwards, and then, above the T_m, the microviscosity decreases to a smaller extent.[51-53] Another example is given by the lateral diffusion of amphiphiles (movement of amphiphiles with respect to the bilayer plane). Lateral movement is diffusion-controlled when the tails are in the liquid-crystalline phase (10^{-11}-10^{-13} m^2 s^{-1}), but it becomes 100-1000 times slower when the tails are in the gel-like state.[50]

The main phase transition temperature is raised upon an increase in length of the alkyl tail. For example, di-*n*-dodecyldimethylammonium bromide has a T_m of 15°C,[54] and dimethyldi-

n-octadecylammonium bromide has a T_m of about 45°C. However, introduction of a double bond in both tails leads to a lowering of the T_m to temperatures below 0°C. The increase in T_m upon an increase in tail size is related to the increasing melting temperature of linear paraffins,[25] which is caused by a stronger crystal packing. Introduction of a double bond leads to a disturbance of the packing (especially in the case of a *cis* double bond). Hence the main phase transition temperature is lowered.



Scheme 1.5. *Schematic representation of a phase transition from a gel-like to a liquid-crystalline state.*

The permeability of the bilayer membranes towards small nonionic organic molecules is high,[55,56] but it is small (10^{-12}-10^{-16} m/s) towards ions, such as chloride ions,[57-59] since it requires the passage of polar groups through an apolar environment.[60] However, hydroxide ions and protons cross the bilayer relatively fast (10^{-6} m/s), despite their ionic character.[59,61-63] Diffusion of water is in the same order of magnitude, and the permeation of H^+ and OH^- ions has been linked to this observation.[64] Whereas water can just diffuse through membranes, random pore formation is required for other ions to cross the membrane. However, it has been suggested that the hydrated ions cross over the membrane rather than dehydrated ions, whereas the smaller hydrated ions pass more easily than larger ones.[65] On the contrary, it has also been shown that anionic vesicles are impermeable towards hydroxide ions,[66] and cationic vesicles only poorly permeable.[67] It has been suggested that permeation of a hydroxide ion requires (slow) permeation of an inert anion in the opposite direction in order to maintain charge neutrality.[68] As a result, the fast permeation of hydroxide ions might be slower depending on the inert ion. The permeation rate constant is estimated to be 10^{-5}-10^{-3} s^{-1}.[69]

Around the main phase transition temperature (and also around other transitions[70]) the membrane is usually more permeable than below or above this temperature.[65,70-76] Addition of single-tailed micelle-forming surfactant enhances pore formation and hence ions diffuse through the membrane more easily.[77]

In accordance with the crossing of small ions, flip-flop (transfer) of amphiphiles from the inner to the outer leaflet and vice versa is also a slow process (k=5· 10^{-5} s^{-1}),[60] but flip-flop is fastest around the T_m.[78,79] Apparently, around the T_m the bilayer packing is not very efficient leading to extensive pore formation or packing defects. This is further exemplified by the observation that around the T_m vesicles are most susceptible to fusion.[80]

1.2 Biological Membranes

1.2.1 General Properties

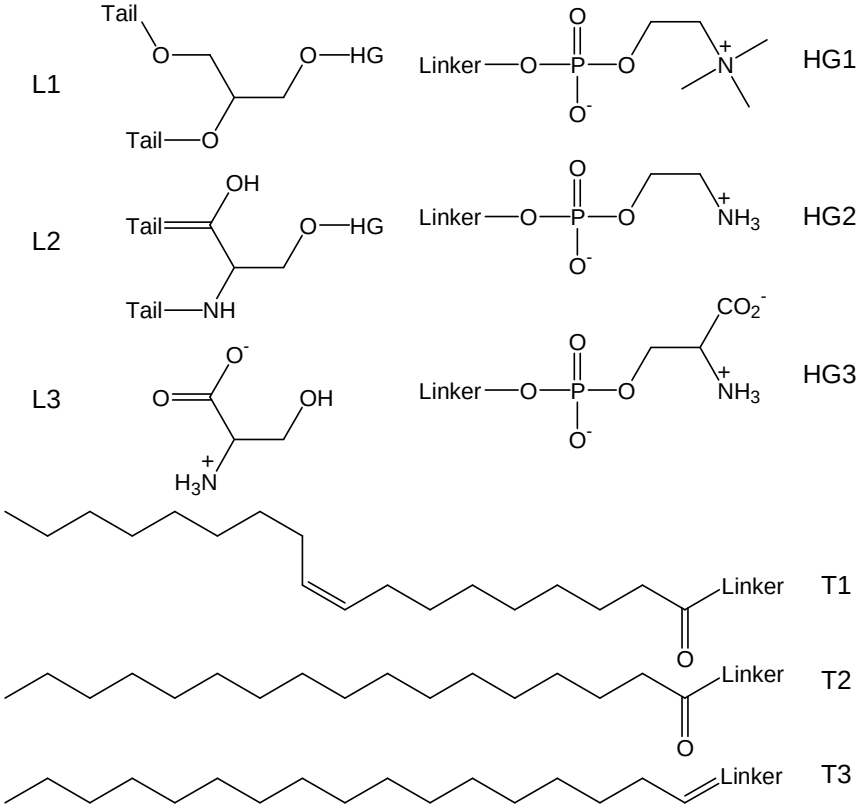
Vesicles are often used as mimics for biological membranes.[32,48,81] However, besides compartmentalising the cell, biological membranes are much more complex and fulfil a number of vital functions for living cells. The type of function and the conditions under which these functions have to be fulfilled, determine the composition of the membranes. Each cell has a number of membranes with each their own composition. For example, the nucleus requires a different type of membrane than the membrane which compartmentalises the cell. A major function of membranes is to carefully control which and how many molecules can enter and leave the cell. These functions rely on strongly specific recognition of those molecules. Failure of this mechanism will lead to malfunctioning of the cell, and in the worst case to cell death.
Through evolution nature has developed membranes that are capable of being adjusted for their task by just using a different composition or by modification of the compounds that make up membranes. For example, whereas most cells only need to survive around neutral pH and ambient temperatures, other cells such as those of the Sulfolobus Acidocaldarius survive at temperatures of around 85°C and pH 2 to 3 by just slightly modifying some of its components.[82-84] Despite this large variety of requirements, all membranes are composed of similar classes of compounds. These classes are (glyco)lipids, steroids and proteins.[85-88]

1.2.2 Components of Biological Membranes

1.2.2.1 Lipids

Despite the wide variety of lipid structures,[85-88] their general structure is similar. They consist of three building blocks. In these building blocks there is a large variety of possible structures, leading to thousands of potential final structures (Scheme 1.6). Interestingly, this is quite similar to the way that most factories construct their products. The use of (simple) standard building blocks makes it easy to design products that meet specific requirements without the need to completely redesign a new product. In this way many different products can be designed from just a few building blocks. It is not only cheap, but also very efficient. A nice example is given by IKEA, where costumers can design their own cupboards from three main building blocks (shelves, vertical framework, sideways framework).[89]
The polar building block of the lipid (head group) is nonionic, zwitterionic or anionic, the apolar building block (tails) typically contains 16 to 24 carbon atoms. In between the tail and the head group is the third building block (linker) that can be glycerol- or serine (sphingomyelin)–based. The name of a phospholipid is usually (but not necessarily) based on the structure of the head group in combination with the linker. For glycerol-based lipids the name is usually derived from the type of head group. Important lipids include phosphatidylcholine (PC) and phosphatidylethanolamine (PE; Scheme 1.6). Zwitterionic and

anionic lipids with a serine-based linker are usually referred to as sphingomyelins (SM). The structure of the tails is not included in the name.

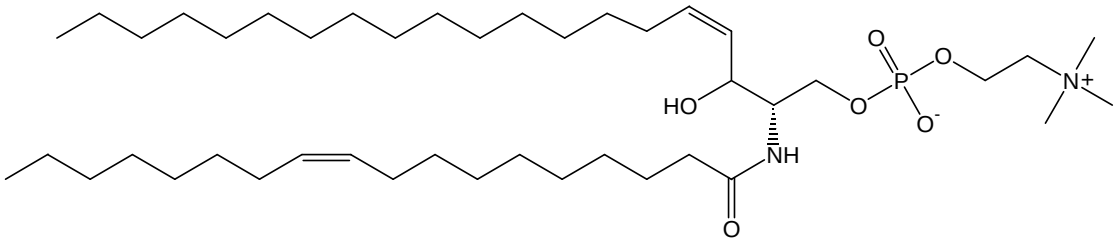


Scheme 1.6. *Some examples of the building blocks of natural occurring phospholipids. Head groups (HG): phosphatidylcholine (PC; HG1), phosphatidylethanolamine (PE; HG2) and phosphatidylserine (PS; HG3). Linkers (L): Glycerol (L1) and Serine-based linker (L2). L3 is the α-amino acid serine. Tails (T): Oleyl (mainly cis; T1) and palmitoyl (T2 and T3). T3 can only be linked to the 1-position in L2. T1 and T2 can be connected to both linkers, but not to the 1-position in L2.*

Nevertheless, the tails are the most important part of the lipid, since they control most of the properties. The tails of phospholipids often contain one or more double bonds. Typically, the number of tails that contains one or more double bonds varies between 50 and 75% (Table 1.1).[90] The tails of SMs (Scheme 1.7) contain considerably fewer double bonds. The origin and function of this observation will be discussed in Section 1.2.3.2. The composition of the tails depends not only on the type of membrane, but also on the head group to which the tails are attached. For example, tails connected to a phosphatidylcholine head group contain 20-30 mol% *n*-hexadecyl tails, whereas the tails connected to a phosphatidyl-ethanolamine contain almost no *n*-hexadecyl tails.

The reason for the presence of double bonds in the tails is that the double bonds maintain the fluid structure of the bilayer, and thereby prevent crystallisation of the tails. For example, vesicles formed from synthetic amphiphiles can break up into fragments below the main phase transition temperature.[91,92] Most of the double bonds have a *cis*-configuration since this has the most pronounced effect on the fluidity. The main phase transition temperature of a phospholipid containing a *trans* double bond is in between the main phase

transition temperature of a saturated tail and one containing a *cis* double bond. In animal cells phospholipids containing both a saturated and an unsaturated tail have the latter tail usually attached to the *sn*-2 position.[86]



Scheme 1.7. *Example of a sphingomyelin (SM).*

Certain bacteria can adapt the amounts of *cis* and *trans* double bonds in their membranes as a response to fluctuations in the external temperature.[93] In extreme cases (high temperatures) bacteria are known to produce membrane-spanning amphiphiles (bola-amphiphiles), that essentially are two phospholipid molecules connected via the tail ends.[84] Other special biological lipid tails include ladderanes.[94]

As discussed, the variety in linkers is limited to only two types of linker. One type is the glycerol-based linker and the other type a serine-based linker. They find their use in their combined action, *i.e.* the fact that both types of linkers are present within the same membrane. Their function will be discussed in more detail in Section 1.2.3.2.

The head groups of phospholipids are nonionic, anionic or zwitterionic. However, they are present in a large variety of structures, which are often closely structurally related (*e.g.* compare PC vs. PE). For each of the individual lipids their function in biological membranes is not clear. Hence, their importance probably comes, just like the linkers, from a combined action in a complex mixture in the membrane.

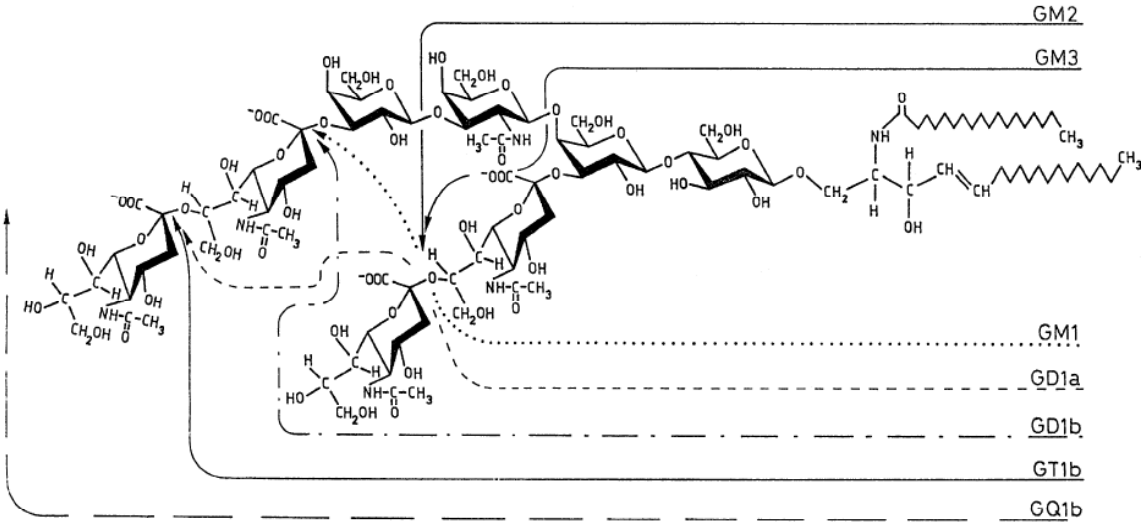
Table 1.1. *Composition of the tails of a few selected cellular membranes (in mol%).*

Tail[a)]	Brain PC	Heart PC	Brain PE	Heart PE	Milk SM
16:0	31	23	5	1	19
18:0	16	6	19	30	3
18:1	39	13	31	4	
18:2	1	43	1	21	
20:4	5	6	19	30	2
22:6	2		9		
other	6	9	16	14	76[c)]
%U[b)]	51	69	71	65	5

[a)] 16:1 means a tail with sixteen carbon atoms and one double bond.

[b)] Mole percentage of unsaturated tails. [c)] Other tails are 20:0 (1%), 22:0 (19%), 23:0 (33%), 24:0 (20%) and 24:1 (3%).

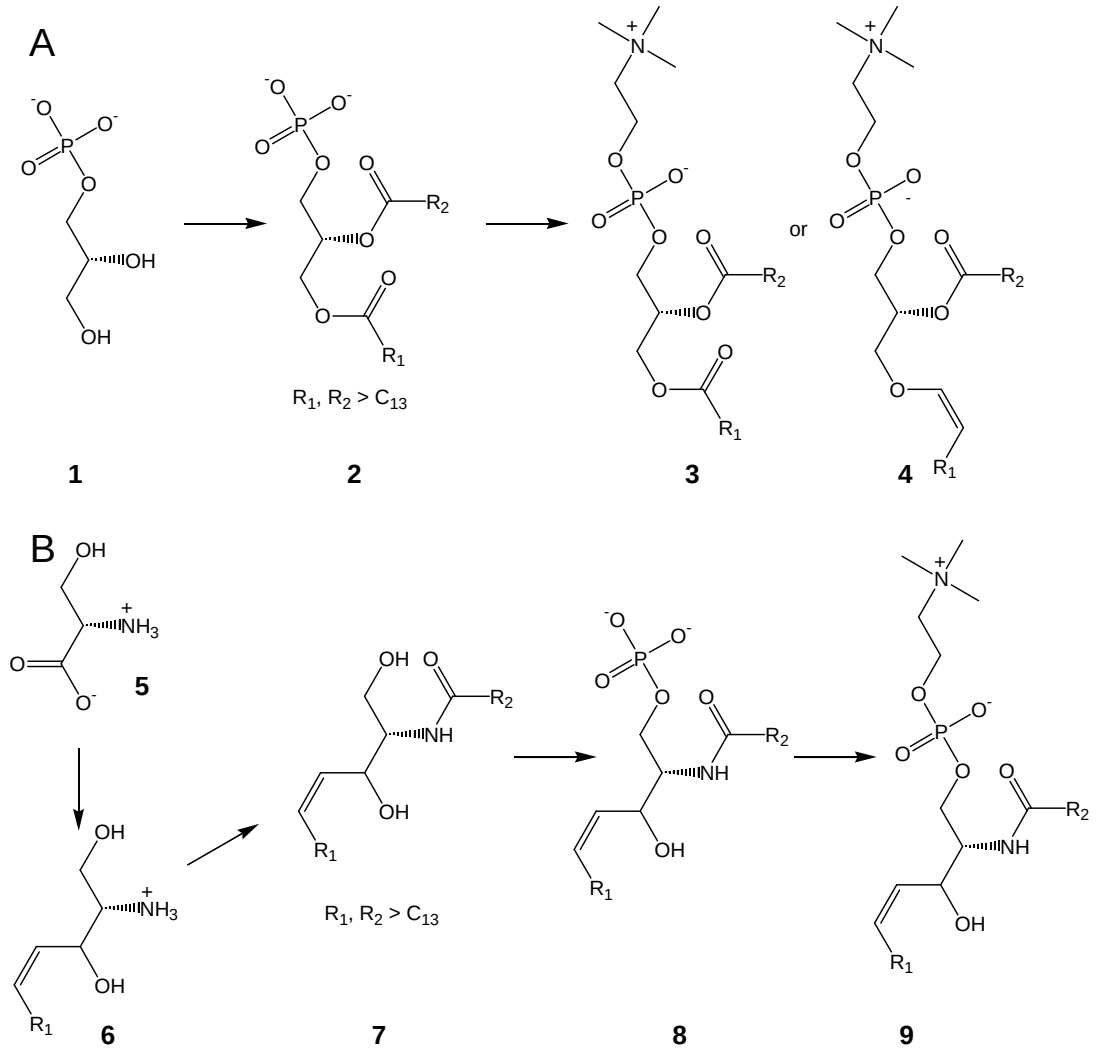
Glycolipids (GL), *i.e.* lipids with a nonionic or anionic oligosaccharide head group, deserve special attention, since they are important for the interactions of a cell with the environment. Their function is broad, ranging from cell adhesion to signal transduction.[96,97] They play a major role in recognition processes by viruses, toxins and bacteria.[98,99] However, many of the details remain not very well understood until now.[100,101] Their behaviour as single component in aqueous solutions has been excellently summarised.[102] In order to allow specificity in their interactions, there is a wide variety of GLs with structural variations in tail, linker, and, most importantly, in the head group. The head group can be as simple as a single sugar, and as complex as a branched oligosaccharide chain (Scheme 1.8). Up to 25 mol% of biological membranes can consist of GLs.[28,88]



Scheme 1.8. *Example illustrating the complex structure of glycolipids. This picture, for example, represents seven different glycolipids. Taken from Tettamanti et. al.*[95]

The biosynthesis of lipid molecules is a complex and multistep and -path process regulated by a series of enzymes. Most of the lipids are synthesised in the endoplasmic reticulum,[103] but also in other places lipids are synthesised, degraded or repaired.[104,105] Briefly, the general procedure of the biosynthesis[85] of glycerol-based phospholipids starts with L-glycerol-3-phosphate (**1**; Scheme 1.9) to which two fatty acid chains are esterified at the hydroxyl groups on the glycerol. The resulting phosphatidic acid (**2**) is then esterified on the phosphoryl group to yield the required phospholipid (**3**). In nerve and muscle cell membranes considerable amounts of plasmalogens (**4**) are present. In these lipids an ether linkage replaces the ester linker on the *sn*-1 of the glycerol.[106]

Biosynthesis of sphingomyelin-based phospholipids starts with serine (**5**) to which a fatty acid is connected via a double bond. The resulting molecule is known as sphingosine (**6**), which is the only naturally occurring cationic surfactant at physiological pH.[107] Additional attachment of another fatty acid via an amide bond leads to ceramides (**7**). Then it is phosphorylated and the appropriate head group is attached, which leads to sphingomyelin (**9**).



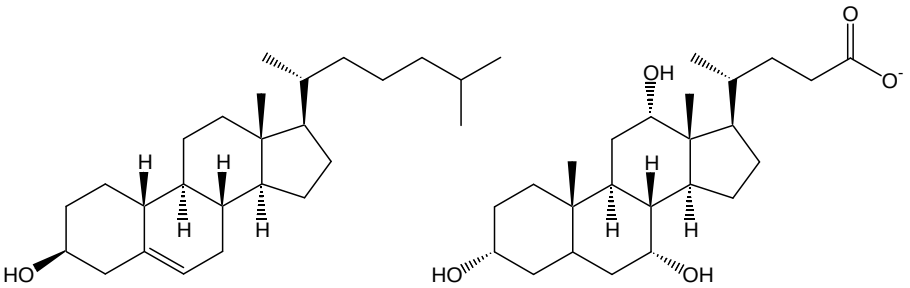
Scheme 1.9. *Simplified representation of the biosynthetic pathway of glycerol-based phospholipids (A) and sphingomyelin-based phospholipids (B).*

The role of ceramides and sphingosine in biological membranes is strongly controversial (over 4000 papers). It has been linked to being messenger for apoptosis (programmed cell death) as a result of sphingomyelin hydrolysis,[108-112] or alternatively to play a role in membrane permeability (channel formation), fusion and other membrane properties.[113] It is also possible that the latter properties are involved in apoptosis.[114]

1.2.2.2 Steroids

Steroids are the second major component in biological membranes. Cholesterol and its derivatives, such as cholate (Scheme 1.10) are the most important contributors. Many studies on cholesterol incorporated into model membranes have been performed, much less attention has been paid to derivatives of cholesterol. However, usually the effect of other steroids is much less pronounced compared to cholesterol.[115] Biological membranes usually contain up to 30 mol% of cholesterol,[87,88] although mole fractions up to 67% have been

isolated from certain membranes.[116,117] Other derivatives of cholesterol that are commonly found in biological membranes include desmosterol, stigmasterol and ergosterol.[118]



Scheme 1.10. *Cholesterol (left) and cholate (right).*

Incorporation of cholesterol improves the chain packing (condensing effect; decrease in the mean molecular area),[119-121] leading to segregation into cholesterol-rich and cholesterol-poor domains above a critical mole fraction.[120,122] In addition, the lateral diffusion of phospholipids decreases upon increasing amounts of cholesterol,[120] as does the permeability towards water[58,123], small ions,[124,125] fluorescent dyes[121,126] and nonionic hydrophilic molecules.[118,127-129] Also non-enzyme mediated flip-flop between inner and outer leaflet is strongly reduced.[79] Interestingly, despite the improved packing, the main phase transition temperatures are hardly affected upon addition of cholesterol.[130-132] However, the transition severely broadens and the enthalpy of the transition decreases at higher mole fractions of cholesterol. This indicates that the corresponding peaks in DSC scans are due to domains poor in cholesterol, since vesicles with a high mole fraction of cholesterol have no transition.[71] In membranes containing several types of lipids, cholesterol prefers to be near those with the lowest phase transition temperature.[131,133,134] Most of the above-mentioned properties of cholesterol do not depend much on the structure of the tails of the lipid.[58,115,131,133,134]

The mechanism through which cholesterol acts is not well understood. Van der Waals interactions alone are not sufficient to explain the above-mentioned observed effects. Therefore, several authors have pointed to the presence of a hydroxyl group in the 3β position of cholesterol-based steroids as being a key factor in their activity.[130] It has been claimed that it forms hydrogen bonds with the carbonyl of the phospholipids, but this appears to be unlikely.[135] The unsaturation in the B-ring has also been taken as the origin of the relatively large condensing effect.[115]

1.2.2.3 Proteins

Proteins control a wide variety of processes and therefore many different proteins are present in membranes. Processes being controlled by proteins include the transport processes between the inner and outer part of the cell and reactions at the surface of the cell that maintain the structural integrity of the membrane. In this thesis, the behaviour of membrane-bound proteins and their interactions with lipids and the cellular environment will not be further discussed.

1.2.3 Features of Biological Membranes

1.2.3.1 Composition

In order to get an idea of the relative abundance of several phospholipids and steroids, the composition of three biological membranes is shown in Table 1.2. The composition of the tails of some phospholipids was already shown in Table 1.1. Being together about 75 mol% of the total lipid/steroid content, it is clear that phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cholesterol are the main components of biological membranes. However, their relative amounts can vary, even if one compares membranes from the same type of cell, but originating from different animals.

Table 1.2. *Composition of a few selected cellular membranes (in mol%).*

Lipid/Steroid	Erythrocyte (human)[86]	Erythrocyte (rat)[85]	Mitochondrion (rat)[85]
PC	25	31	48
PE	22	15	24
PS	10	7	2
SM	18	8.5	4
Cholesterol	25	24	4
GL[a)]	n.d.[b)]	3	-
PI[c)]	n.d.[b)]	2	10
PA[d)]	n.d.[b)]	0.1	1
others[e)]	-	-	13

[a)] Glycolipid. [b)] Not determined. [c)] Phosphatidylinositol. [d)] Phosphatidic acid .
[e)] Others include phosphatidylglycerol and cardiolipin.

1.2.3.2 Lateral and Transverse Asymmetry

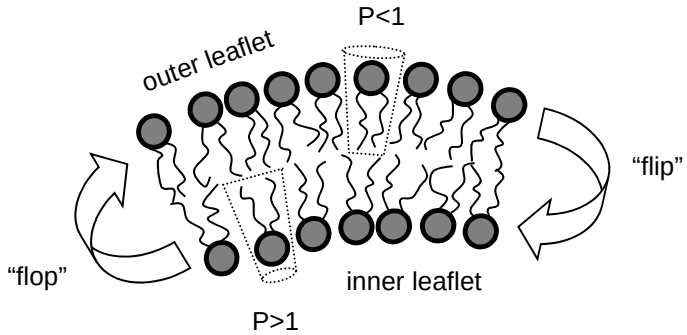
Knowledge of the composition of biological membranes is important. However, understanding their local organisation is crucial if one wants to understand the details of how biological membranes operate. For example, cholesterol and sphingomyelins (SM) are known to form liquid-ordered domains ("rafts") in membranes, to which certain proteins prefer to bind.[135,136] These rafts, that are insoluble towards detergents, such as Triton X-100, are domains in which the lipids are ordered, similar to the gel phase, but they undergo fast diffusion as in the liquid phase. The driving force for this domain formation has been related to the relatively high abundance of saturated tails and the hydrophobic nature of the sphingosine linker in SMs, and the possibility to form hydrogen bonds via the amide functionality in the linker. Hydrogen bonds with cholesterol seem to play an important role as well,[137-140] whereas this type of interaction does not occur with glycerol-based phospholipids.[135] This additional interaction with SMs is required to explain the high concentration of cholesterol in these rafts, since cholesterol prefers to be in the presence of

phospholipids with the lowest T_m (Section 1.2.2.2). Lipids with the lowest T_m can be found in the liquid-disordered matrix formed by unsaturated phospholipids, rather than in the rafts formed by saturated SMs. Using a variety of techniques, the size of these domains has been estimated to be between 10 and 1000 nm in diameter. However, this large distribution of reported sizes indicates a lack of detailed knowledge of these domains rather than an understanding of the origin of and driving forces for raft formation.[141]

Several authors have attempted to unravel potential driving forces of segregation other than raft formation in model membranes.[142-150] The results are often in disagreement with each other, which makes understanding difficult. However, little debate exists over the observation that when a phospholipid with two identical tails is mixed with a phospholipid with tails which are at least four carbon atoms longer or shorter segregation into domains occurs.[151,152] The effect is not only related to the mismatch in size of the tail, but it can also be a result of the two lipids having different phases (liquid-crystalline versus gel-like).[153] The presence of cholesterol in such mixtures has led to controversy with respect to whether it strengthens segregation,[154-157] or diminishes it.[158-160]

In conclusion, segregation can occur as a result of several parameters. However, in model membranes segregation strongly depends on the structure of the lipids and the presence of additives in the bilayer. Its mechanism is unclear. In biological membranes raft formation plays a crucial role. However, the mechanism of raft formation remains only poorly understood.[136] The biological need for raft formation is related to protein sorting and cell signalling. A excellent review including more detailed information on raft formations and its function has been written by Brown.[135]

Another key factor in the functioning of biological membranes is the asymmetric distribution of lipids over the inner and outer leaflet. In order to perform their role in the interaction of cells with their environment glycolipids reside preferably in the outer leaflet of membranes.[161] In addition, also SM and PC are mainly found in the outer leaflet, whereas the amine-containing glycerophospholipids, such as PS and PE, are mainly found in the inner leaflet.[162]



Scheme 1.11. *Schematic representation of flip-flop and the spatial requirements for phospholipids in the inner and outer leaflet of a cell membrane.*

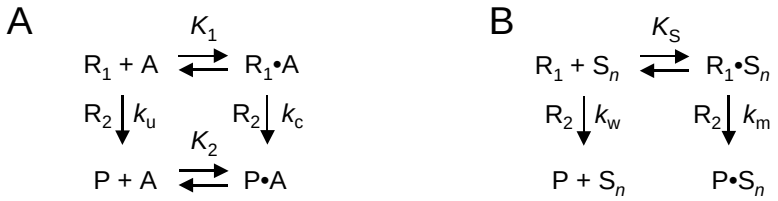
Since spontaneous flip-flop is slow (several hours to days depending on the composition of the membrane),[60,79,163] especially in cholesterol-containing membranes,[79] nature makes use of three classes of proteins to promote and control flip-flop.[164,165] At this point it should be noted that, as depicted in Scheme 1.11, the transport of a lipid from the outer leaflet to the inner leaflet is called "flip", and that the reverse process is called "flop". Two of the protein classes involved, flippase and floppase, selectively transport lipids from one leaflet to the

other in an ATP-driven process. Flippase very selectively transports PS from the outer to the inner leaflet, but floppase seems to be less selective in its transport of lipids to the outer leaflet. A non-ATP-driven class of proteins, scramblase, randomly and non-selectively transports lipids between either leaflets. Scramblase is required in biological membranes since lipid biosynthesis typically occurs in only one of the leaflets. For example, PS, PC and PE are mainly synthesised in the inner leaflet of the endoplasmic reticulum.
Besides protein-mediated asymmetry, the asymmetric distribution is also reasonable from a physical point of view. Lipids, such as PE, have a packing parameter that is larger than 1 (Section 1.1.2.1; Scheme 1.11), which is required for having an efficiently packed inner leaflet.[166] In the outer leaflet lipids require on average a packing parameter smaller than 1, which is the case in PC and SM rich leaflets. Due to their large head group, glycolipids have a relatively small packing parameter. GM1 (Scheme 1.8), for example, forms micelles when dissolved in water.[167] These observations indicate that the asymmetry in biological membranes is, at least partially, maintained by a difference in the packing parameter.[168]

1.3 Catalysis of Organic Reactions in Aggregates

1.3.1 General Considerations

Catalysis of organic reactions by micelles has been studied for a long time.[169-171] In several of these early studies it was pointed out that these systems were model systems for biological membranes or even enzymes. Unfortunately, a model to fit the experimental data was absent until 1967 when Menger and Portnoy developed such a model (Scheme 1.12B) to account for the hydroxide-ion catalysed hydrolysis of several esters in both the aqueous and micellar phase of anionic and cationic micelles.[172] They adopted a model (Scheme 1.12A) used to calculate the catalysed and uncatalysed rate constant for the acetolysis of 2,4,7-trinitro-9-fluorenyl-*p*-toluenesulfonate in the presence of phenanthrene with which it forms a 1:1 complex.[173] Unfortunately, the model made it only possible to fit the data of solutions for which inhibition of the reaction was observed, since binding of hydroxide ions to the micellar surface was not taken into account. However, despite this limitation it was possible to prove that the hydroxide-ion catalysed reaction did not take place in anionic micelles.[174] Later several authors refined the theory to include binding of more than one organic substrate or hydrophilic ions.[175-186]



Scheme 1.12. *Schematic representation of the models used to describe catalysis by a 1:1 complex (A) and by micelle-formation. R_1 and R_2 are the reactants and P the products. K_1, K_2 and K_S are binding constants to A (non reactive additive), and S_n (micelle), respectively. k_u, k_c, k_w and k_m are the uncatalysed, catalysed, aqueous and micellar rate constant, respectively.*

In general, two major effects lead to catalysis of bimolecular reactions in micellar and vesicular aggregates.[184] The first effect is entropic in nature and results from substrate-aggregate binding. Charged micelles and vesicles provide a good environment for hydrophobic and oppositely charged molecules to bind, thereby increasing the chances of two substrates to meet and react because the effective reaction volume is reduced.[187] Particularly when one of the two reactants can bind as a counterion to the aggregate, efficient catalysis is found, since the concentration of head groups in the Stern layer of micelles is in the order of 1 to 5 M.[188,189] By contrast, when only one of the two reactants binds to the aggregate, inhibition is observed.

The second effect results from the decreased local polarity at the micellar and vesicular binding sites compared to water. Of course the latter effect is only beneficial when the organic reaction is accelerated in less polar environments. The decrease in polarity is due to a decrease in water concentration and the presence of the (apolar) tails of the amphiphile in the polar-apolar interface. This decrease in polarity is partially counteracted by the presence of polar (charged) head groups. The dielectric constant (at 25°C) of the micellar or vesicular surface is *ca.* 32, which is much lower that the dielectric constant of water (78).[190] Upon binding to aggregates, the reactants are (partially) dehydrated. As a result, the difference between the Gibbs energy of the initial state and the activated complex can change and hence the rate constant in the aggregate is affected. An increase in vesicular or micellar rate constant is particularly observed when one of the reactants is a hydrophilic counterion. The importance of dehydration for these types of reactions has been exemplified by gas phase experiments. It has been revealed that in S_N2 reactions dehydration of the ion is the major factor in the reactivity which may lead to rate accelerations up to 10^{17}.[191-196] Upon binding of one water molecule to the anion the rate constant drops 35%, whereas the heat of the reaction goes from -232 kJ $mole^{-1}$ when water is absent to +3 kJ $mole^{-1}$ when 3 water molecules hydrate the anion.[192]

In reactions where the hydroxide ion is one of the reactants, attention should be drawn to the abnormal behaviour of hydroxide ions with respect to their bulk aqueous behaviour. For example, their mobility is high compared to other anions (similar relationship as between protons and other cations). Although the (complex) mechanisms for this high mobility are different for protons than for hydroxide ions, this special behaviour is a result of the structural similarity of these ions with water.[197-200] Little is known about the implications of this behaviour for micellar and vesicular catalysis where the hydroxide ion is one of the reactants.

The exact binding location of organic molecules in aggregates has long been under debate. However, it is now well established that fully apolar molecules, such as hydrocarbons, bind deeply into the bilayer or micelle. Introduction of any polar group leads to preferential binding at the polar-apolar interface.[201-204] In addition, the orientation of the reactive centre of the reactants with respect to the second reactant in the aggregate has been studied as well.[205-208] For example, if the reactive centre of a substrate is orientated towards the inner core of a bilayer or micelle the reaction with hydrophilic ions is slowed down or inhibited, rather than catalysed. However, in cases where the substrate is small, there is no restriction or preference in orientation or movement within aggregates.

For clarity and completeness, we will briefly address the most important features of the kinetic (mathematical) model as shown in Scheme 1.12B. Slightly different models exist, such as the one derived by Berezin[175-179] and the one derived by Romsted,[181] but they are

based on the same principles and lead to similar results. The model takes into account two pseudophases, an aqueous one and an aggregate pseudophase, in which the reaction proceeds with an aqueous rate constant (k_w) and the aggregate rate constant (k_{agg}), respectively. Therefore, the model is called the pseudophase model. Distribution of the reactants among the pseudophases can be calculated in two different ways, either using partition coefficients P (distribution is a function of the aggregate volume), or the binding constants K (distribution is a function of the aggregate concentration).[209]

When one of the reactants is a hydrophilic counterion in competition with inert ions to bind to the apolar (but charged) pseudophase, the pseudophase model with ion exchange (PPIE) can be used. This model considers the total fraction of bound reactive and inert ions to be independent of the concentration of surfactant. However, there is competition between the counterions to bind to charged surfaces. In systems with only one highly hydrophilic ion, such as hydroxide, cyanide and fluoride ions, these assumptions fail, and alternative models to account for ion binding have to be used.[183,210-213] In addition, charged surfactants with these types of counterions behave differently compared to "normal" counterions. For example, micelles with relatively low aggregation numbers, high cmc and/or a (variable) low counterion binding are formed,[214-220] or micelles are formed from surfactants that usually aggregate into vesicles.[221-223]

After considering the above-mentioned remarks, the observed rate constant can be calculated as a location- and rate-average of the reaction proceeding in the aqueous pseudophase and the aggregate pseudophase.

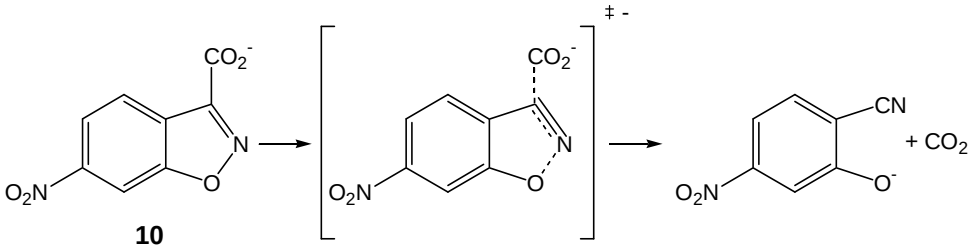
The reaction in the aggregate pseudophase should be corrected for the volume of the aggregate, since the reaction in the aggregate pseudophase only takes place in the volume of the aggregates, whereas the surfactant concentration is expressed as a function of the total volume. This correction is always somewhat troublesome, since the reaction does not necessarily have to take place in the whole volume of the aggregate.[224]

Finally, special care has to be taken with respect to parameter compensation, since this can play a major role in the analysis of the kinetic data.[183,225-229] A more detailed analysis of parameter compensation and the mathematical description of the pseudophase model can be found in Chapter 3.

1.3.2 Vesicular Catalysis

Ever since the design and synthesis of the first synthetic bilayer-forming amphiphiles by Kunitake[230,231] and Fendler[61] many reactions have been performed in the presence of vesicles derived from synthetic amphiphiles. One of the first examples of vesicular catalysis involves the unimolecular decarboxylation reaction of 6-nitrobenzisoxazole-3-carboxylate (6-NBIC; **10**) under basic conditions (Scheme 1.13). This reaction has been studied in detail both in a variety of solvents and in micellar solutions.[232-236] The rate constant strongly depends on the polarity of the medium (an increase in polarity increases the rate constant) and the ability to form hydrogen bonds to the carboxylate group (hydrogen bonds decrease the rate constant). For example, in the series water, methanol, ethanol, 1,4-dioxane, DMSO and HMPA[237] the rate constant relative to the rate constant in water is 1, 34, 135, 5400, 1.3×10^6 and 10^8, respectively. In vesicles formed from di-*n*-alkyldimethylammonium bromide

(n=1-7; Scheme 1.4B) the rate constant of the decarboxylation reaction is higher with respect to the aqueous rate constant, but also with respect to the rate constant in CTAB micelles (n-hexadecyltrimethylammonium bromide; Scheme 1.3A).[235] In micelles formed from CTAB the catalytic rate acceleration relative to water (k_{agg}/k_w) amounts to *ca.* 54 at 30°C. Rough estimates indicate that in cationic vesicles the catalytic rate acceleration is about 10^2 below the main phase transition temperature and 10^3 above the main phase transition temperature.[238,239] The origin of the difference in the catalytic rate acceleration below and above the main phase transition temperature is difficult to pinpoint, but probably arises not so much from a change in polarity,[240] but rather comes from a change in the mobility of water molecules near the amphiphile head groups.[241,242] Phospholipid liposomes catalyse the decarboxylation reaction of **10** about 4-15 times less efficient than synthetic cationic vesicles.[239]



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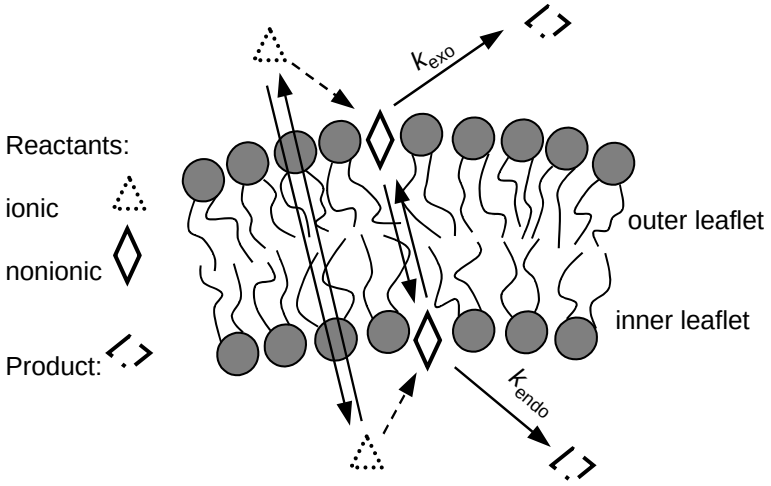
Scheme 1.13. *Unimolecular decarboxylation of 6-nitrobenzisoxazole-3-carboxylate (6-NBIC;* ***10****).*

For bimolecular reactions in the presence of vesicles several parameters have to be taken into account that are not important for bimolecular reactions in micelles. These parameters include the phase of the tails, the permeability of the membrane towards both reactants, the rate constant of the inner leaflet (endovesicular rate constant; Scheme 1.14) compared to the rate constant of the outer leaflet (exovesicular rate constant) and the rate constant as a function of the size of the vesicles.

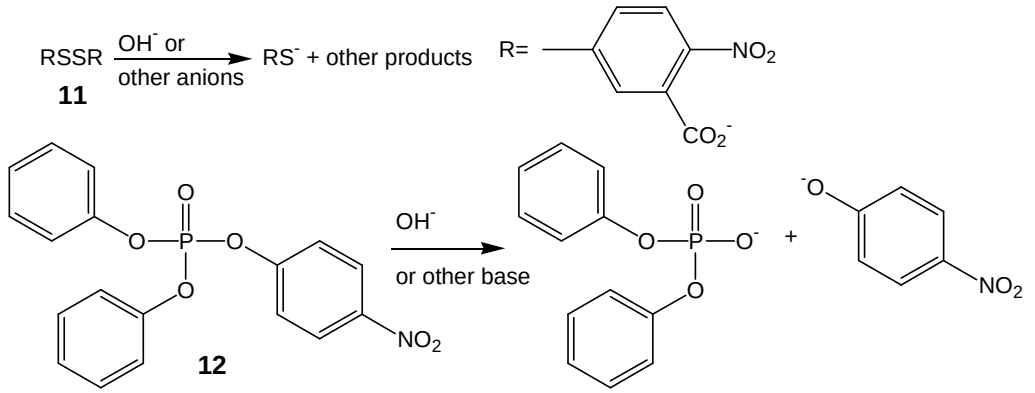


Scheme 1.14. *Schematic representation of the distribution of a nonionic and an ionic reactant in a vesicular solution over the inner and outer leaflet. The reaction proceeds with the rate constant k_{exo} in the interface of the outer leaflet and with k_{endo} in the interface of the inner leaflet.*

It is especially important to address the influence of the phase of the tails, since, besides its influence on the vesicular rate constant, some of the bilayer properties depend on the phase of the tails (Section 1.1.2.3) For example, the permeability is a function of the phase of the tails. Especially around the T_m an increase in the permeability occurs.

Permeability-dependent rate constants have been addressed in detail by Moss *et al.*.[243-245] They studied the reaction of **11** with a series of anions in vesicles formed from dimethyldi-*n*-octadecylammonium chloride at 25°C. At this temperature the tails are in the gel-like state. Using various thiolate anions it was possible to follow the permeation-rate dependent observed endovesicular rate constant relative to the non-permeation-dependent observed exovesicular rate constant. Similar permeation-rate dependent observed rate constants have been measured for another series of reactions.[246-248] Discrimination between the endovesicular rate constant and the exovesicular rate constant was lost by decreasing the size of the tails with two CH_2 units, since then the rate constants were measured around the T_m of di-*n*-hexadecyldimethylammonium bromide.[69,78,244,245,249] Around the T_m, the rate of permeation of the thiolate ions through the bilayer is faster than the rate constant, and hence permeation is no longer the rate-determining step for the endovesicular reaction. The permeation-dependence was also lost when single-tailed surfactants were added.[67] This dependence was also lost after addition of 0.2 wt% of 1-hexanol which lowers the T_m to 25°C.[245]

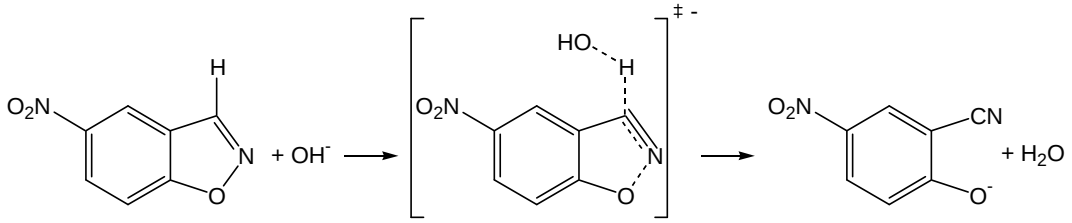
Care has to be taken in assigning changes in absorbance to endo- or exovesicular rate constants, as is exemplified by the following example. In the vesicle-catalysed[250] alkaline hydrolysis of **12** a slow and a fast process were observed (Scheme 1.15).[251-253] The rate of deprotonation of a fluorescent dye indicated that permeation of the bilayer by hydroxide ions was fast on the time scale of the reaction and therefore the two processes were assigned to the observed endo- and exovesicular rate constant. It was found that the observed exovesicular rate constant is about 15-30 times faster than the observed endovesicular rate constant. However, they also observed that at most 20% of the reaction took place in the endovesicular phase. Later, the slow process was assigned to a slow reorganisation of vesicles after placing them under osmotic stress (a result of the kinetic experiment), which led to a small and slow change in turbidity.[254]



Scheme 1.15. *The reaction of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB; **11**) and p-nitrophenyl diphenyl phosphate (**12**) under alkaline conditions.*

The phase of the tails alone on the rate constant were studied in the following examples where permeation of reactants did not play a role. As discussed above, the unimolecular

decarboxylation of **10** is about ten times faster above the T_m, as was measured by reducing the size of the hydrophobic tails.[235] The hydroxide-ion catalysed reaction of **13** (Scheme 1.16; Kemp elimination) and the imidazole-catalysed hydrolysis of an ester are also faster when the amphiphile is above the T_m.[255,256] When the amphiphiles are structurally varied in order to perform the kinetic experiments above the T_m at a constant temperature, usually the tails are decreased in length, since a decrease in tail length leads to a lowering of the T_m. In general, a decrease in tail length leads to a slightly higher local polarity, which usually leads to a lower vesicular rate constant. Instead a higher vesicular rate constant is mostly observed. Hence, this indicates that the small change in local polarity is not so important, but that an increase in fluidity of the tails is usually more important.
Alternatively to structural variation of the amphiphile, the influence of the phase of the tails can be studied by construction of an Arrhenius plot (log(*k*) versus 1/*T*). Upon increasing the temperature the Arrhenius plot of the imidazole-catalysed hydrolysis of an ester, described above, deviates from linearity around the T_m of the amphiphile, whereas above and below the T_m the experimental data points are on a straight line. Both the intercept and the slope of the straight lines change at the T_m. This illustrates that both $\Delta^{\ddagger}H^{\circ}$ and $\Delta^{\ddagger}S^{\circ}$ are affected by changing the phase of the tails.[257] However, the hydroxide-ion catalysed hydrolysis of the same ester shows no break in the Arrhenius plot. The alkaline hydrolysis of a different ester is relatively slowed down above the T_m.[258] These results show that not in all cases a change in fluidity of the membranes leads to a beneficial increase in the rate constant. In addition, the effect of a change in fluidity of the tails leads to a complex change of interactions as is indicated by changes in both $\Delta^{\ddagger}H^{\circ}$ and $\Delta^{\ddagger}S^{\circ}$.



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Scheme 1.16. *General base-catalysed deprotonation reaction of 5-nitrobenzisoxazole (5-NB;* ***13****)*

Different rate constant for the inner and outer leaflet were subject of study by Chaimovich *et al.*[259] By selectively binding of **11** to the inner leaflet of dimethyldi-*n*-octadecylammonium chloride vesicles, they were able to follow the reaction with hydroxide ions to both leaflets independently. It was found that **11** did not leak out of the vesicles in a period of 24 h, which is reasonable considering the phase of the tails and the fact that **11** is a dianion. They did not find a marked difference between the endo- and exovesicular rate constant, indicating that the inner and outer leaflet are not significantly different in structure.
Few studies concern vesicular catalysis as a function of the size of the vesicle. However, in two of these studies differently-sized vesicles were prepared by comparing sonicated vesicles and ethanol-injected vesicles.[39,253] As discussed in section 1.1.2.3, the addition of ethanol can induce changes in the properties, and hence comparison these two types of vesicles is dangerous. If the influence of ethanol in the vesicular solution is neglected, it can be concluded that the trend in the alkaline hydrolysis and thiolysis of *p*-nitrophenyl octanoate

as a function of vesicle size (between 22 and 285 nm) depends on the counterion (bromide or chloride) of the cationic amphiphile.[39] With increasing vesicle size the rate constants increased for vesicles with a chloride counterion, but decreased for vesicles with a bromide counterion. The hydrolysis of **12** is slowed down by increasing the vesicle size (between 50 and 160 nm).[253] In a third study, the rate of Diels-Alder reactions was measured as a function of the vesicle size (30 and 300 nm).[261] The vesicles were prepared by sonication (30 nm) or hydration of an amphiphile film and subsequent extrusion (300 nm). No significant change in the rate constant was observed.

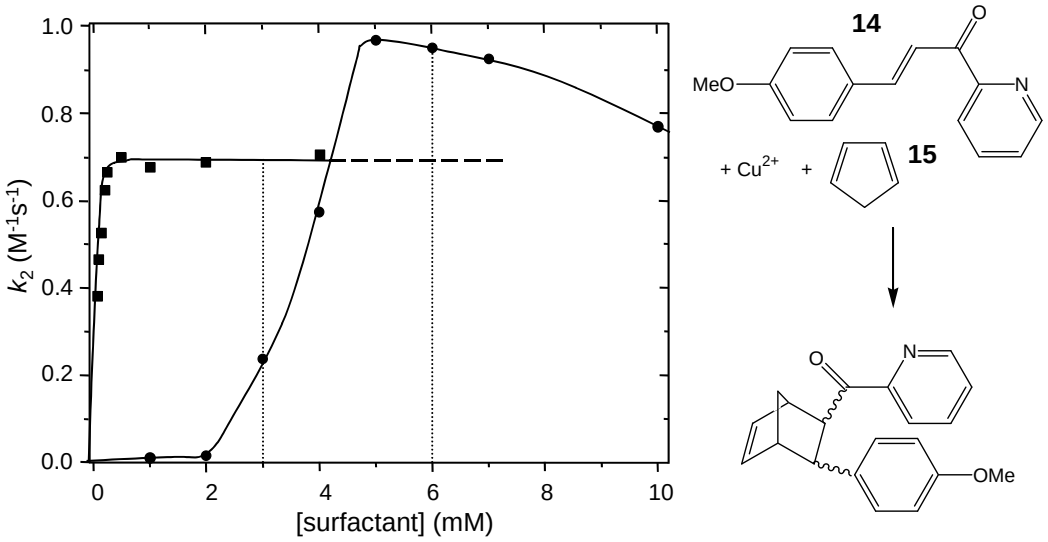

Figure 1.1. *Example of complications arising from comparing observed rate constants for the Diels-Alder reaction of* ***14*** *and* ***15*** *in the presence of* Cu^{2+}*, catalysed by micelles (●) and vesicles (■). The rate constants presented are the observed (pseudo)-first-order rate constants divided by the concentration of* ***15****. Taken from Rispens et al.*[260]

In many of the studies observed rate constants at a certain amphiphile concentration are compared with each other. In these cases care has to be taken, since the observed rate constant depends both on the distribution of the reactants between the aqueous and vesicular phase and the vesicular and aqueous rate constants as discussed in 1.3.1. In summary, the distribution of the reactants over the two pseudophases is a function of both the binding constant of the reactants and the amphiphile concentration. The binding constant and the vesicular rate constant depend on the structure of the amphiphile and temperature. Hence, due to these complex dependences, it can be ambitious to compare observed rate constants. For example, several authors have compared observed rate constants in micellar and vesicular solutions, leading to the conclusions that vesicles are better catalysts,[235,245,253,256,262,263] or that micelles are better catalysts.[246,247,264,265] An example is given in Figure 1.1, where the observed rate constants of the micelle- and vesicle-catalysed Diels-Alder reaction of **14** and **15** at two different surfactant concentrations are compared.[260] It can be concluded that both vesicles and micelles are better catalysts depending on the concentration of surfactant at which this comparison is made. At 3 mM surfactant the ratio of the observed micellar rate constant and observed vesicular rate constant ($k_{obs,mic}/k_{obs,ves}$) is 0.4, whereas at 6 mM this ratio is 1.4.

Finally, examples of the wide variety of reactions that can be catalysed in the presence of vesicles are given. These include peptide bond cleavage,[264] bromination,[265] ester hydrolysis,[266] ester thiolysis,[267] DNA hydrolysis,[268] oxidation,[269] electron transfer reactions,[270-274] diazo coupling reactions,[262,275] arenediazonium ion decomposition,[276] styrene polymerisation[277] and nitroso group transfer.[278] It has been proven that in these cases the observed rate constants were higher than those in water, but no new insights into the mechanism of vesicular catalysis were obtained.

1.4 Aim and Outline of this Thesis

Due to its relative simplicity, both in handling and interpretation (discussed above), micellar catalysis is more widely discussed in the literature than vesicular catalysis.[185] However, vesicles are much more akin to biological membranes than micelles. This does not necessarily mean that studying vesicular catalysis leads to further insight into processes in biological membranes. So far, most studies involving vesicular catalysis have been mainly carried out in single-component systems, *i.e.* amphiphiles are the only hydrophobic component in the system. By contrast, biological membranes have a complex composition with many different components. Studies of properties of model membranes involving phospholipids, sphingomyelins and steroids are important and have a high biological relevance, but understanding of the observations is often troubled by multiple possible interactions between the components. This is exemplified by the extensive discussion about the origin of raft formation, and which interactions are driving forces for this phenomenon. Therefore, limiting the number of possible interactions between the components, but introducing small structural variation within a series of additives, might reveal some of the factors that are important in catalysis occurring in biological membranes.

Based on the considerations above, we decided to examine the influence of the composition of the vesicle bilayer on vesicular catalysis. A suitable probe reaction for these vesicles is the hydroxide-ion-catalysed deprotonation reaction of **13** (Kemp elimination). The mechanism of the E2 Kemp elimination reaction has been well studied,[279-281] and efficient catalysis has been found in micelles, vesicles, (synthetic) anti-bodies and modified cyclodextrines.[256,282-291] Important factors for significant rate enhancements were found to be desolvation of the base, a hydrophobic binding site and a decrease of the reaction volume as a result from binding of the two reactants to a hydrophobic binding site in an aggregate.

Dimethyldi-*n*-octadecylammonium chloride was selected as vesicle forming amphiphile, since its properties have been well studied (including vesicular catalysis; Section 1.3.2). In addition, it is structurally simple, which is beneficial for understanding interactions with its environment. Similar considerations were made in selecting a wide variety of additives. This choice of additives allows relatively easy correlation between the obtained results and the structural variation of the additives.

In vesicles, both reactants permeate fast through the bilayer and the two reactants are dependent on different parameters to bind to bilayers. Whereas **13** binds to vesicles due to hydrophobic interactions, the hydroxide ion only binds as a result of the electrostatic attraction. This introduces more independent parameters for different types of interactions.

Chapter 1 includes a brief introduction of hydrophobic interactions with the focus on aggregate formation. Specific attention is paid to vesicles and their general properties. Then the components and behaviour of biological membranes are discussed. In the last part vesicular catalysis as a mimetic medium for biological membranes is discussed, including parameters that are important in vesicular catalysis.
Chapter 2 describes the influence of the additives that are used throughout Chapter 3 to 6 on vesicle properties, such as vesicle shape and size, the main phase transition temperature and membrane polarity.
Chapter 3 describes the kinetic model that is derived in order to study the influence of the additives on vesicular catalysis. Parameters obtained from the analysis include the vesicular rate constant, the binding constant of the kinetic probe to the vesicle, the counterion binding to the charged head groups of the cationic amphiphile and the competition between the anions in solution to bind to the vesicles. The limitations of the kinetic model are discussed as well. The influence of the addition of anionic double-tailed amphiphiles on the probe reaction is delineated. The anionic amphiphiles introduced into the vesicles have either two decyl tails, or a decyl and an octadecyl tail.
Chapter 4 deals with the influence of long linear mono- and dihydric alcohols in the vesicles on the probe reaction. The monohydric alcohols include *n*-decanol, *n*-octadecanol and oleyl alcohol. The dihydric alcohol is 3-*n*-octadecyloxy-propane-1,2-diol (batyl alcohol). Despite their structural similarity each alcohol has its own specific effect.
Chapter 5 discusses the effects on the catalysis of the presence of ethylene glycol units in the Stern region. The ethylene glycol units are attached via two different hydrophobic anchors. One anchor is a hexadecyl tail, the other is based on a (cationic) SAINT-2 amphiphile (*N*-methyl-4-(dioleylmethyl)pyridinium chloride).
Chapter 6 reports the influence of glucose and maltose, anchored into the bilayer, on the catalytic decomposition of **13**. These additives were chosen as mimics for glycolipids. Glycolipids play an important role in the structural integrity of biological membranes and the communication of cells and their environment.
Chapter 7 reviews the most important conclusions from this thesis and, based on that, suggests new research projects in the field of vesicular catalysis and its relevance for understanding biological membranes.

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